Title: MULTIPLEX NUCLEIC ACID REACTIONS
Inventors: Chee et al.
Filing Date: July 15, 2003
Serial No.: 10/620,852
Attorney Client-Matter No.: 67234-015
Tel: 858-535-9001

OIPE JC11
JAN 20 2004
PATENT & TRADEMARK OFFICE

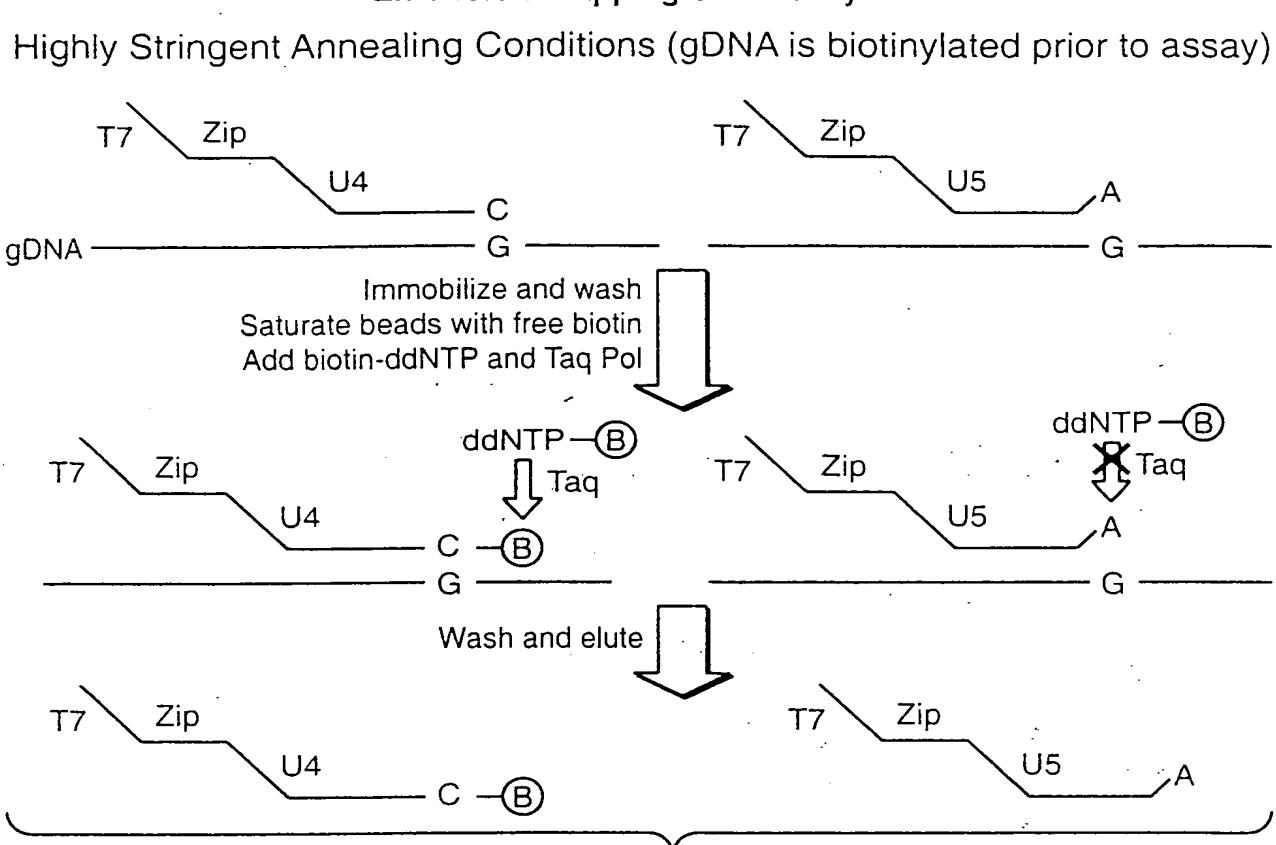


FIG._1A

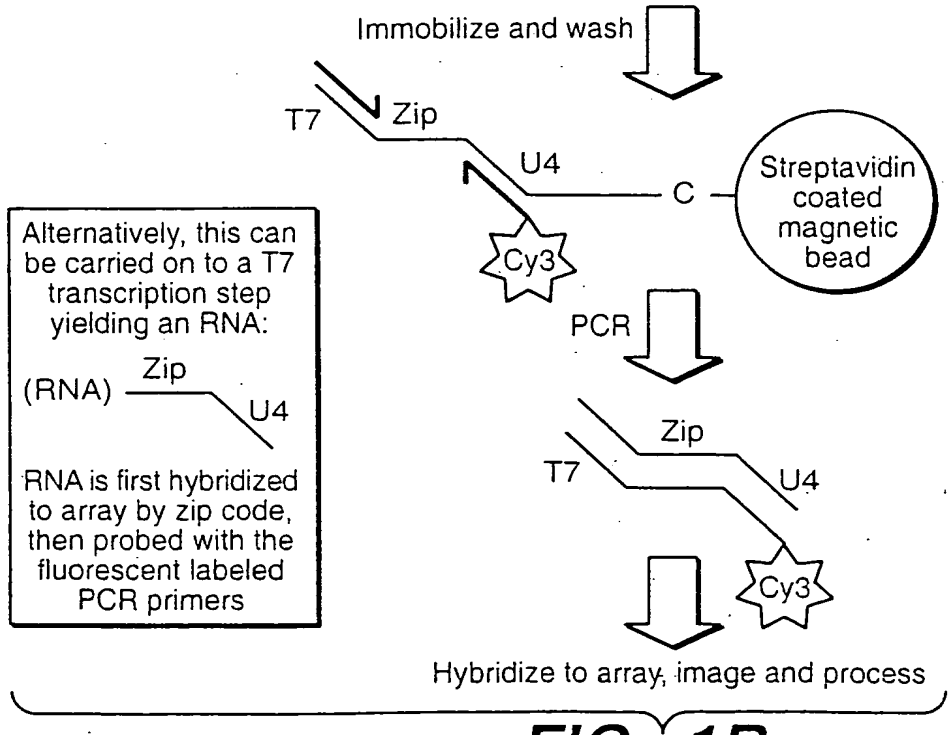


FIG._1B

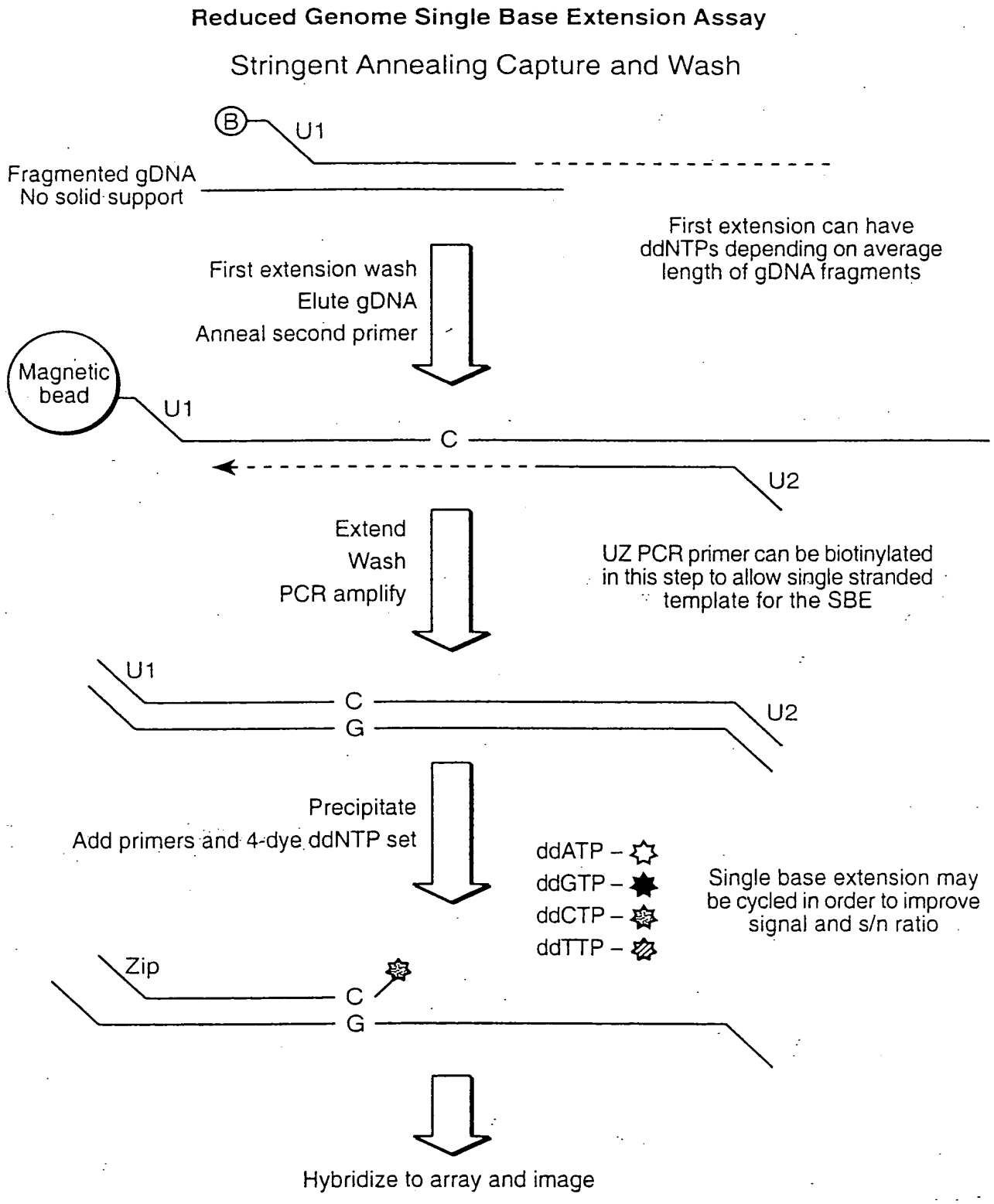

Reduced Genome Single Base Extension Assay
Stringent Annealing Capture and Wash
B
U1
Fragmented gDNA
No solid support
First extension can have ddNTPs depending on average length of gDNA fragments
First extension wash
Elute gDNA
Anneal second primer
Magnetic bead
U1
C
U2
Extend
Wash
PCR amplify
UZ PCR primer can be biotinylated in this step to allow single stranded template for the SBE
U1
C
G
U2
Precipitate
Add primers and 4-dye ddNTP set
ddATP –
ddGTP –
ddCTP –
ddTTP –
Single base extension may be cycled in order to improve signal and s/n ratio
Zip
C
G
Hybridize to array and image

FIG._2

Title: MULTIPLEX NUCLEIC ACID REACTIONS
Inventors: Chee et al.
Filing Date: July 15, 2003
Serial No.: 10/620,852
Attorney Client-Matter No.: 67234-015
Tel: 858-535-9001

Complexity Reduction and Multiplex Assay

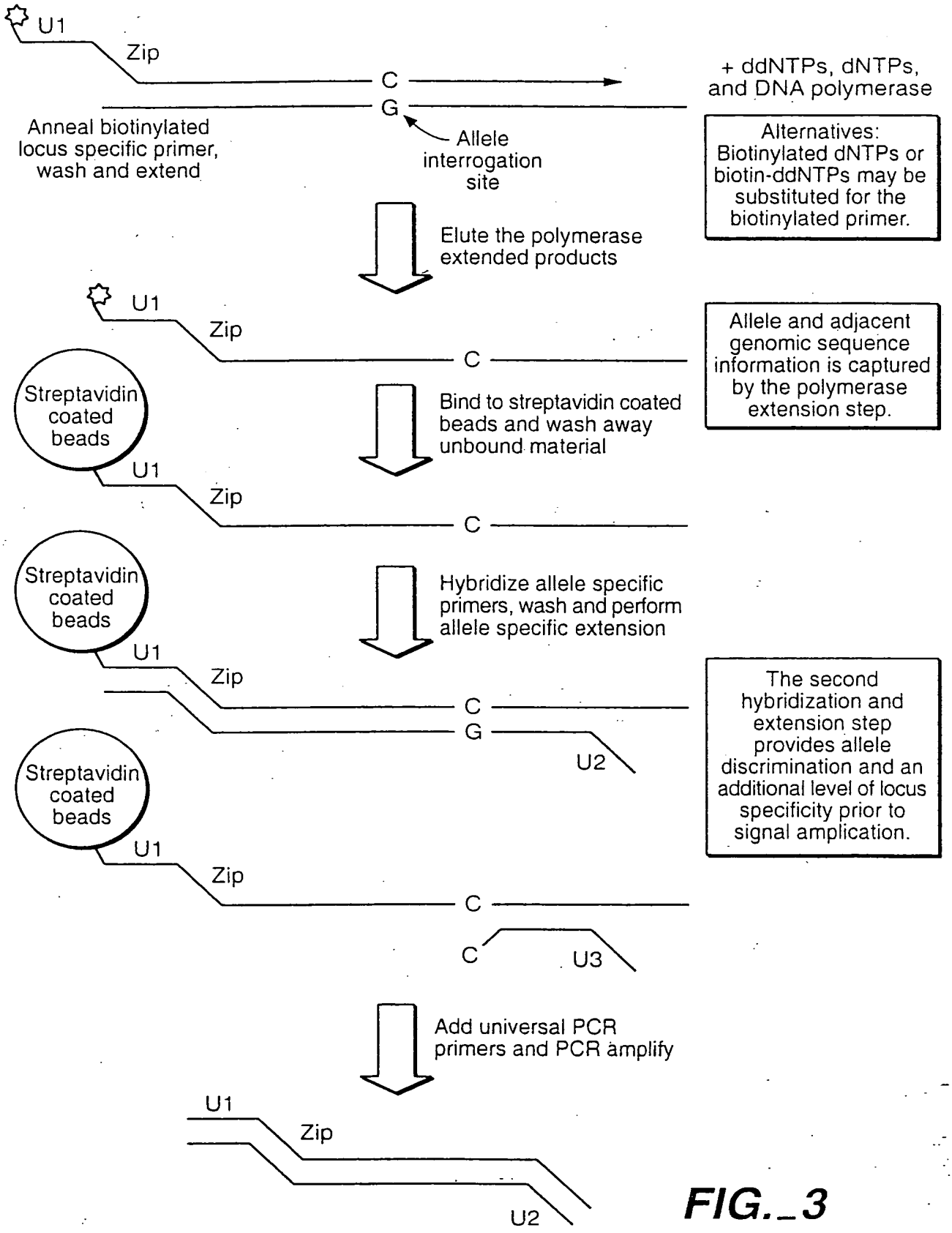


FIG._3

Complexity Reduction and Multiplex Assay

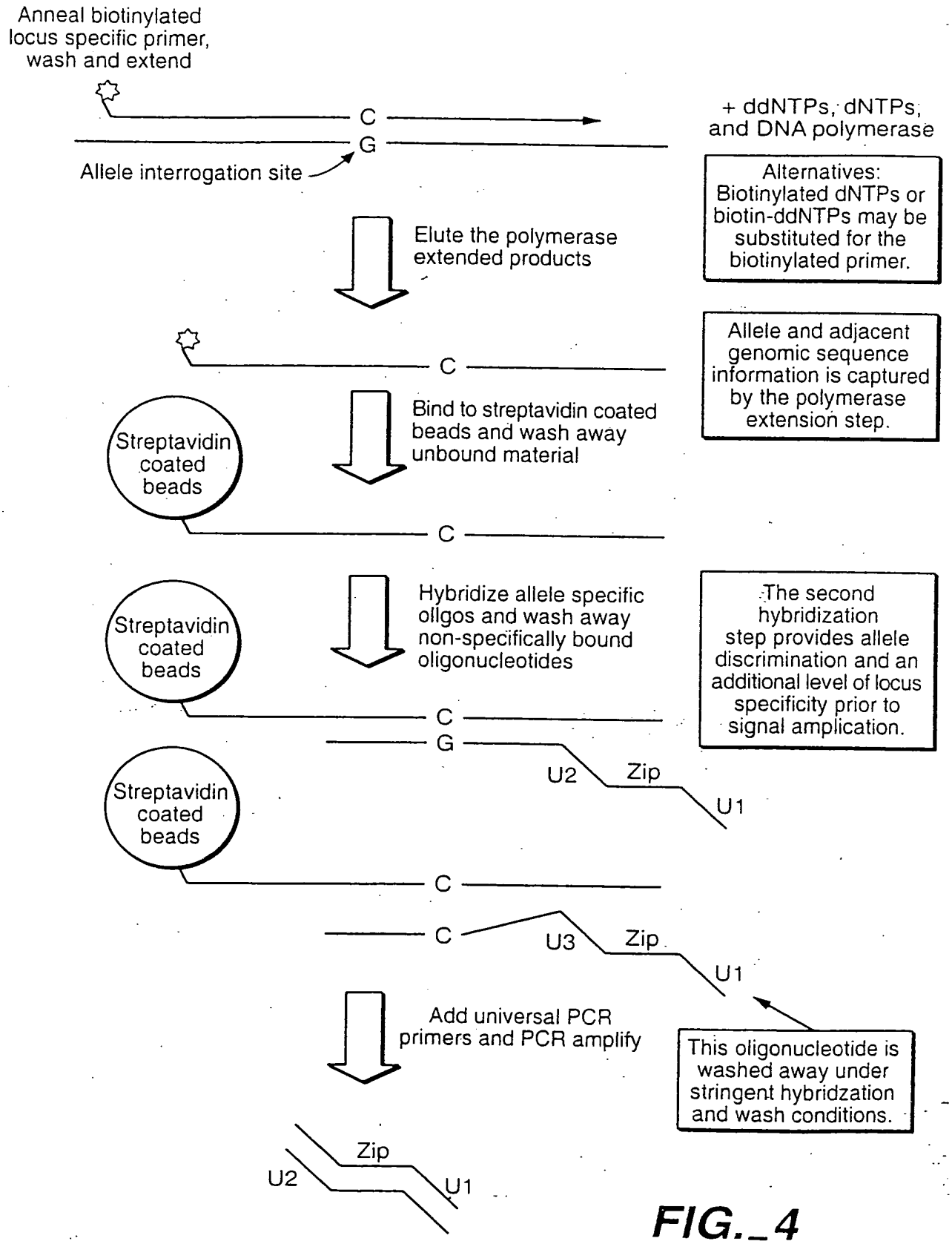


FIG._4

Complexity Reduction and Multiplex Assay

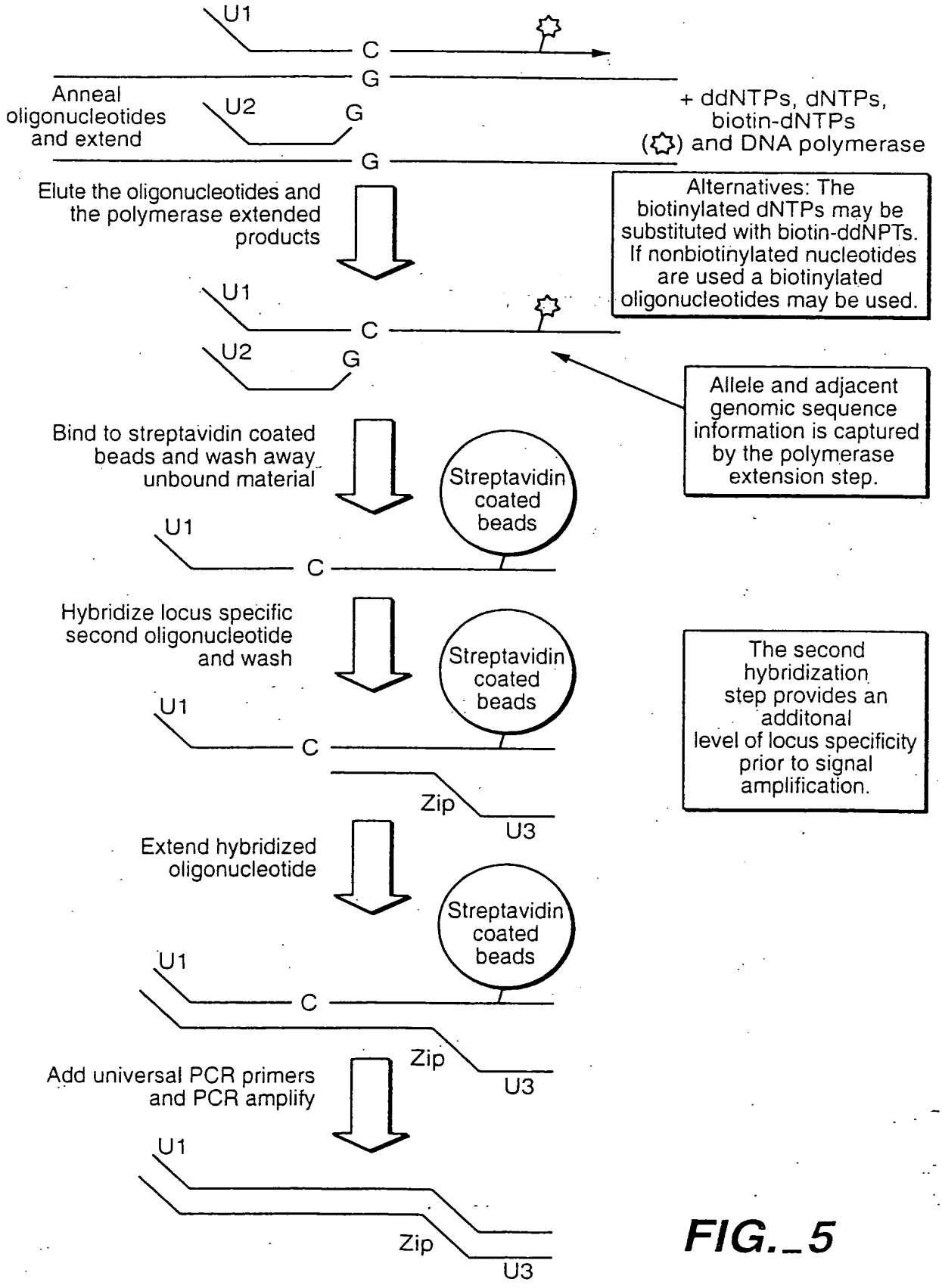


FIG._5

Complexity Reduction and Multiplex Assay

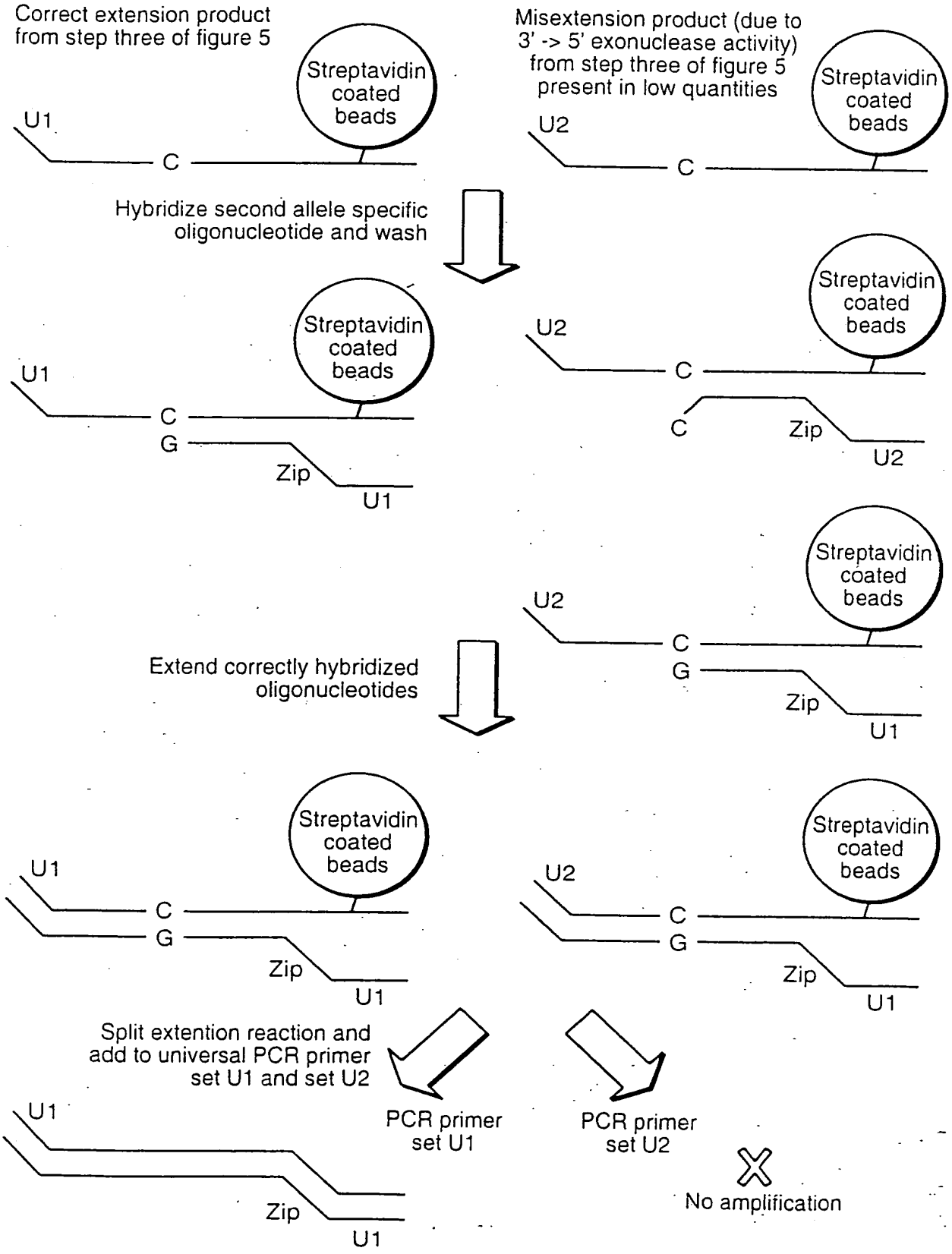


FIG._6

Solid Phase Locus-Specific Primer Extension

Starting material is immobilized, single stranded universal PCR product. There are several ways to generate this.

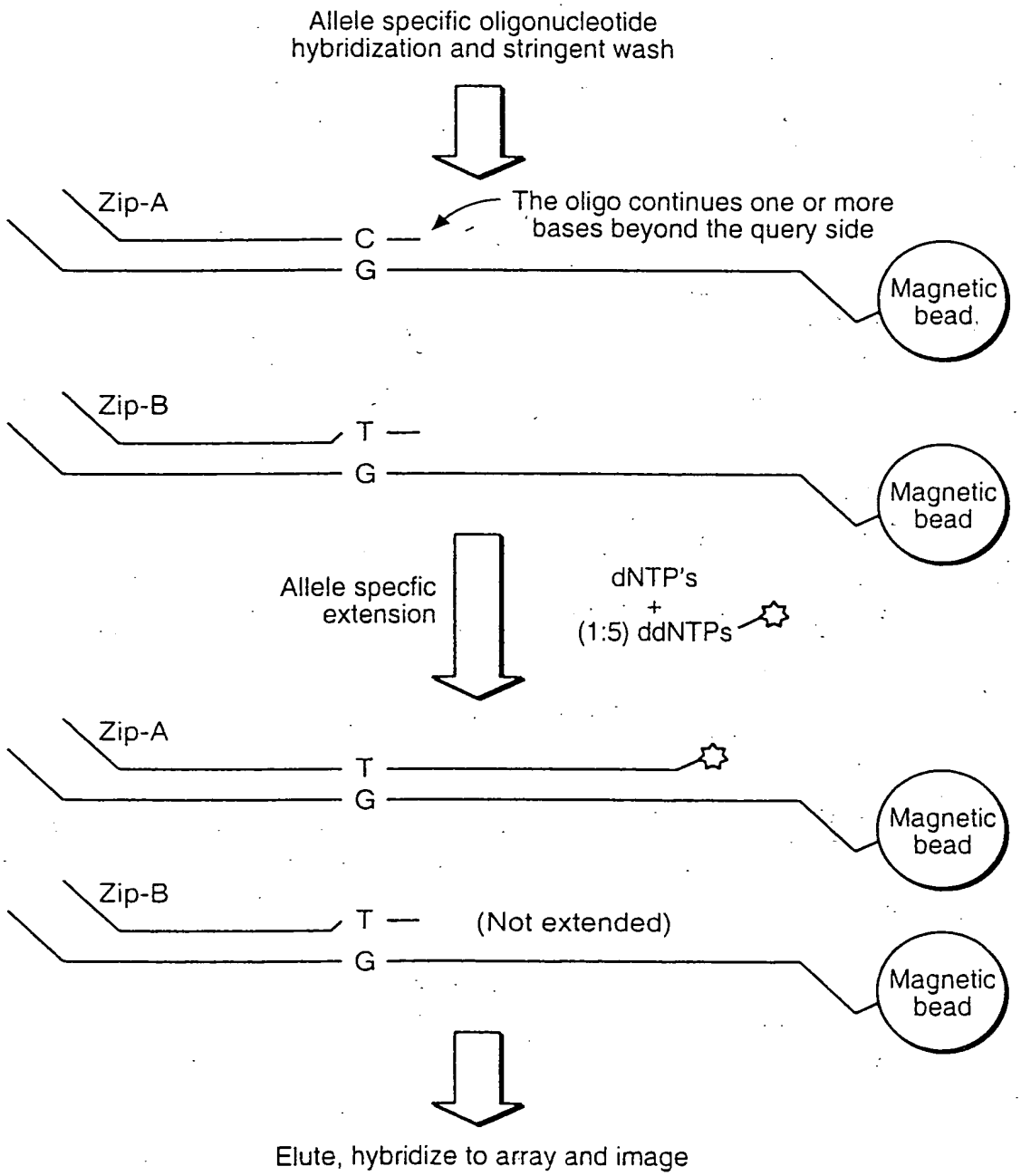


FIG._7

Alternate Labeling Scheme for Primer Extension (High Signal)

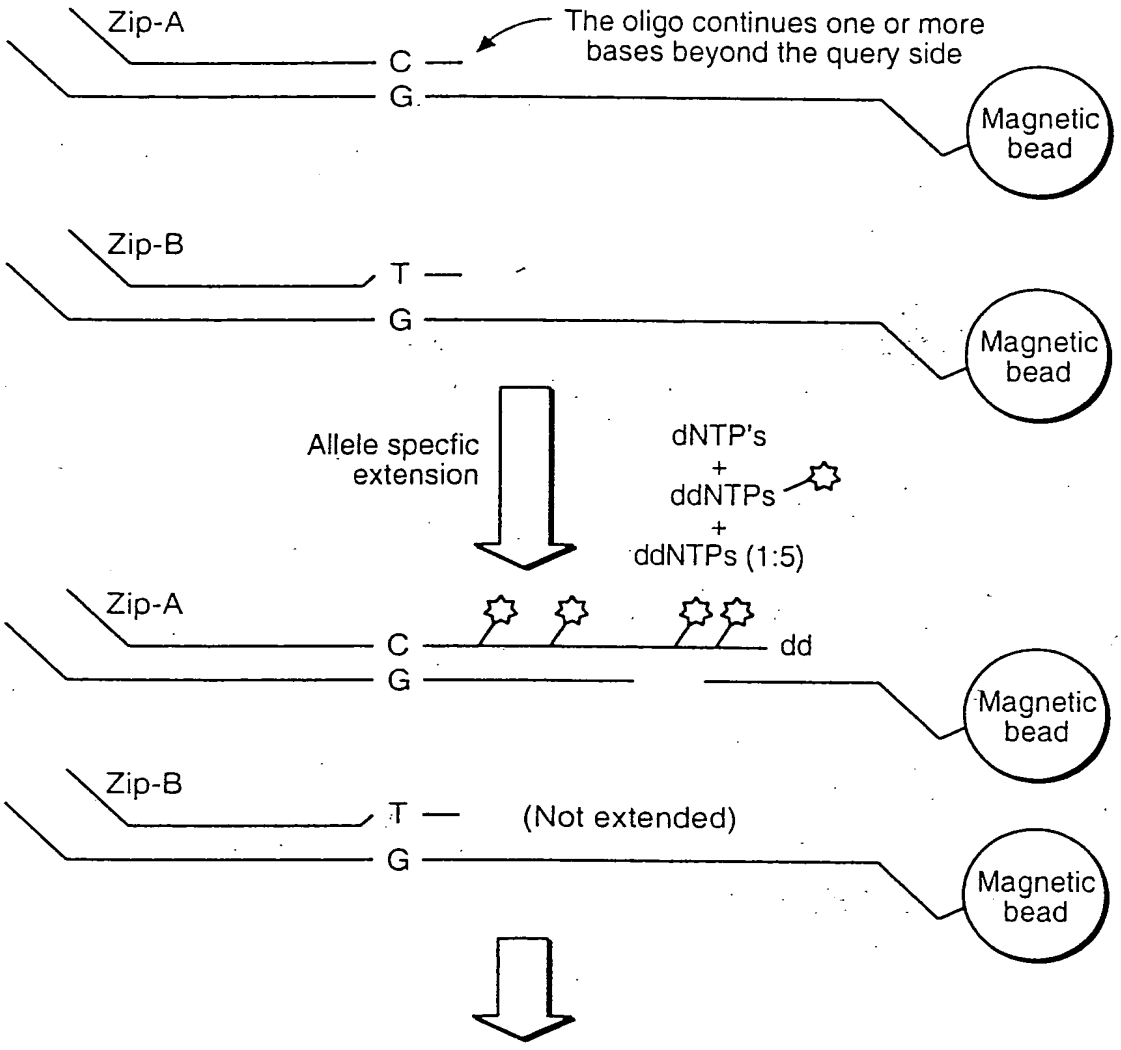


FIG._8

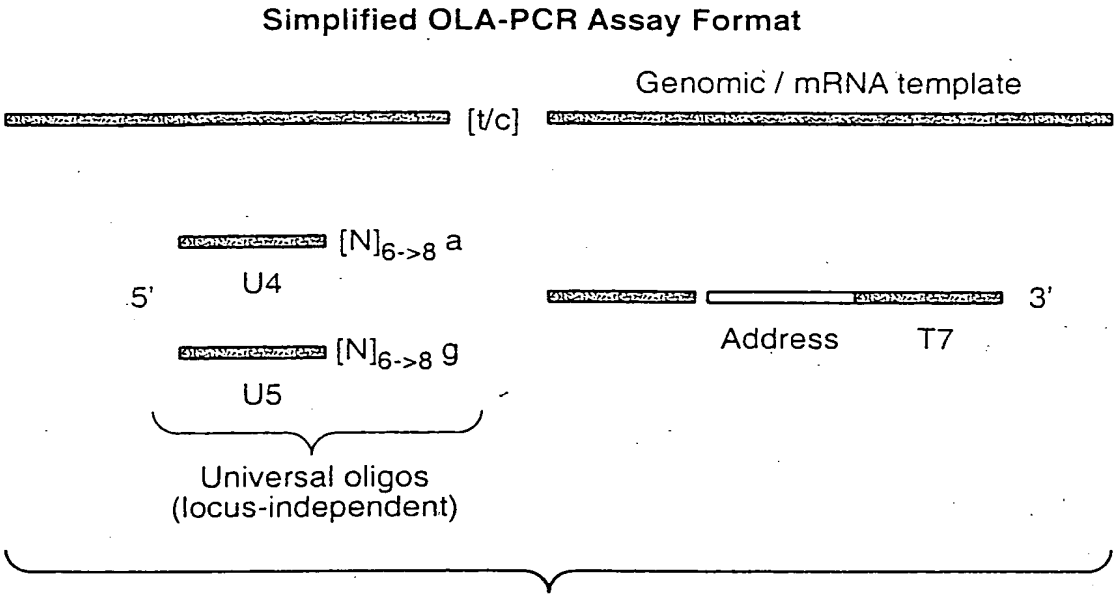

Simplified OLA-PCR Assay Format
Genomic / mRNA template
[t/c]
$[N]_{6->8}$ a
5'
U4
$[N]_{6->8}$ g
U5
Universal oligos
(locus-independent)
3'
Address
T7

FIG._9

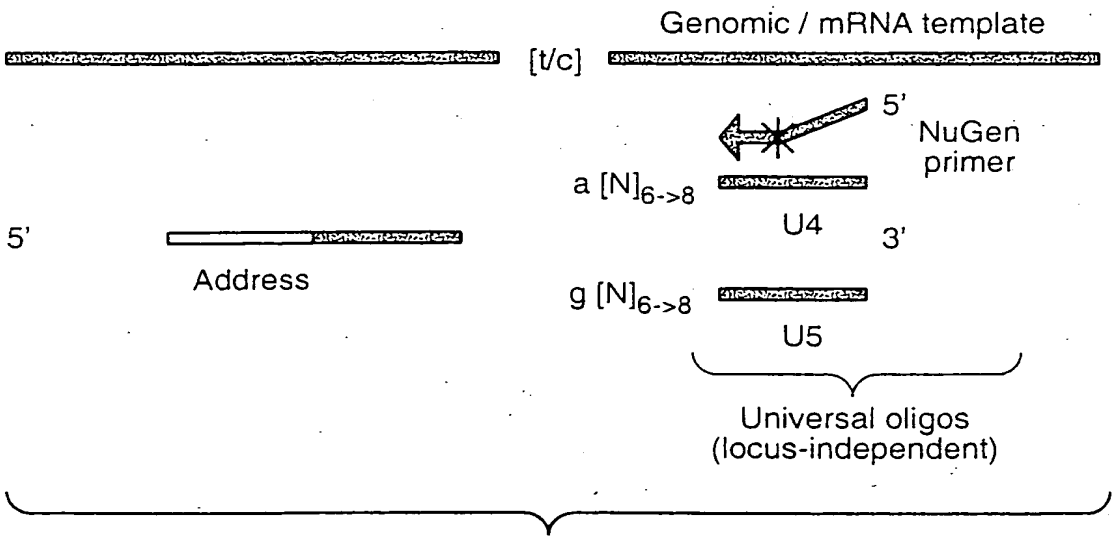

"Reverse" S-OLA-PCR Assay Format
Genomic / mRNA template
[t/c]
5'
NuGen
primer
a $[N]_{6->8}$
U4
3'
5'
Address
g $[N]_{6->8}$
U5
Universal oligos
(locus-independent)

FIG._10

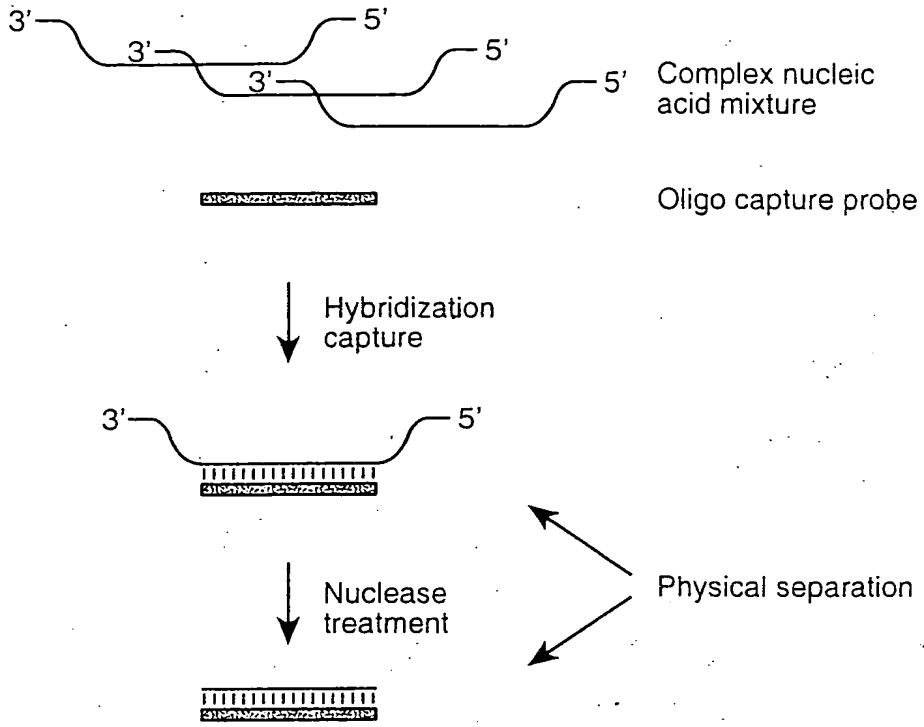

3'
5'
3'
5'
3'
5'
Complex nucleic acid mixture
Oligo capture probe
Hybridization capture
3'
5'
Nuclease treatment
Physical separation

FIG._11

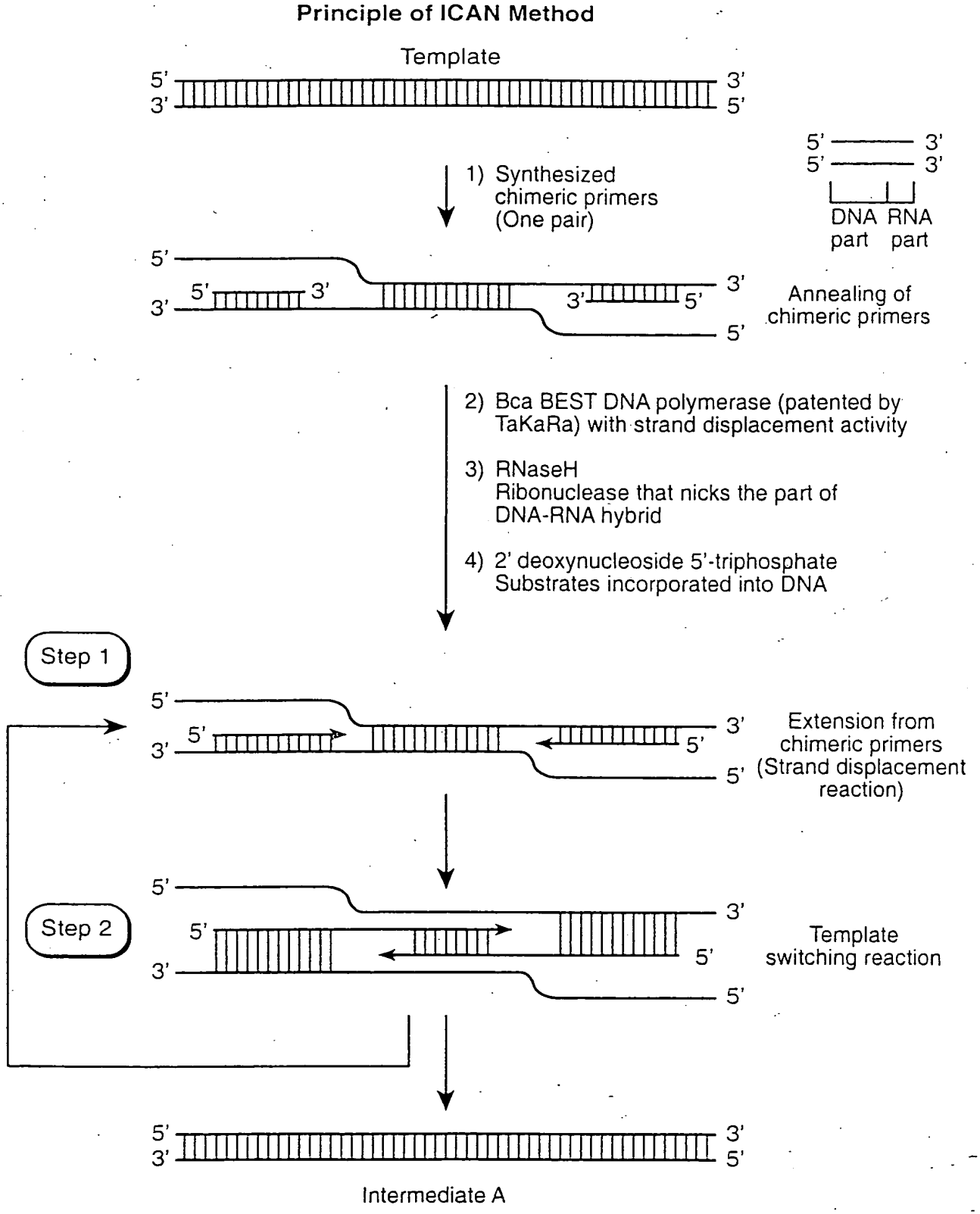

Principle of ICAN Method
Template
5'
3'
3'
5'
5' 3'
5' 3'
DNA part
RNA part
1) Synthesized chimeric primers (One pair)
Annealing of chimeric primers
2) Bca BEST DNA polymerase (patented by TaKaRa) with strand displacement activity
3) RNaseH
Ribonuclease that nicks the part of DNA-RNA hybrid
4) 2' deoxynucleoside 5'-triphosphate
Substrates incorporated into DNA
Step 1
Extension from chimeric primers (Strand displacement reaction)
Step 2
Template switching reaction
Intermediate A

FIG._12A

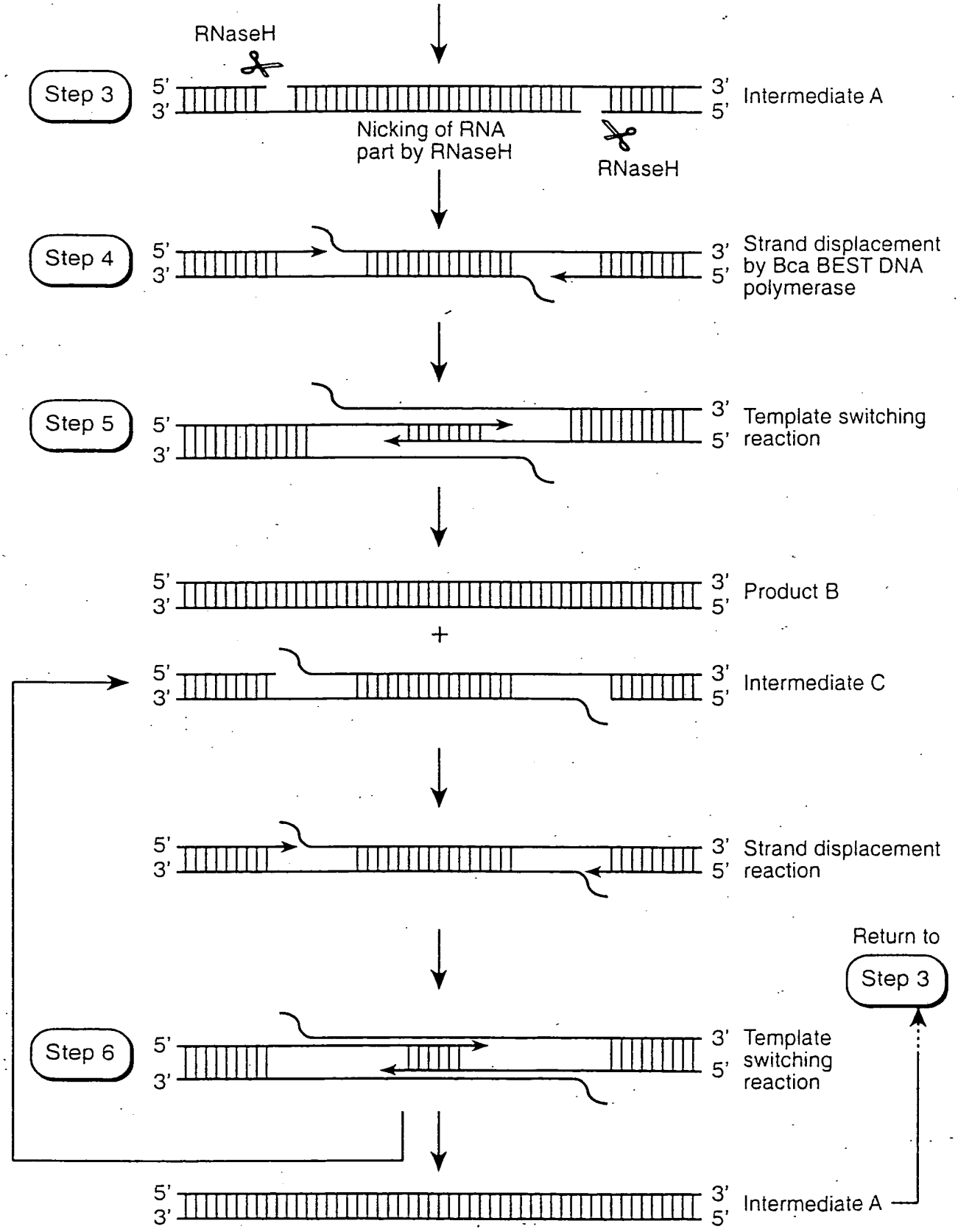

RNaseH
Step 3
5'
3'
3'
5'
Intermediate A
Nicking of RNA part by RNaseH
RNaseH
Step 4
Strand displacement by Bca BEST DNA polymerase
Step 5
Template switching reaction
Product B
+
Intermediate C
Strand displacement reaction
Return to
Step 3
Step 6
Template switching reaction
Intermediate A

FIG._12B

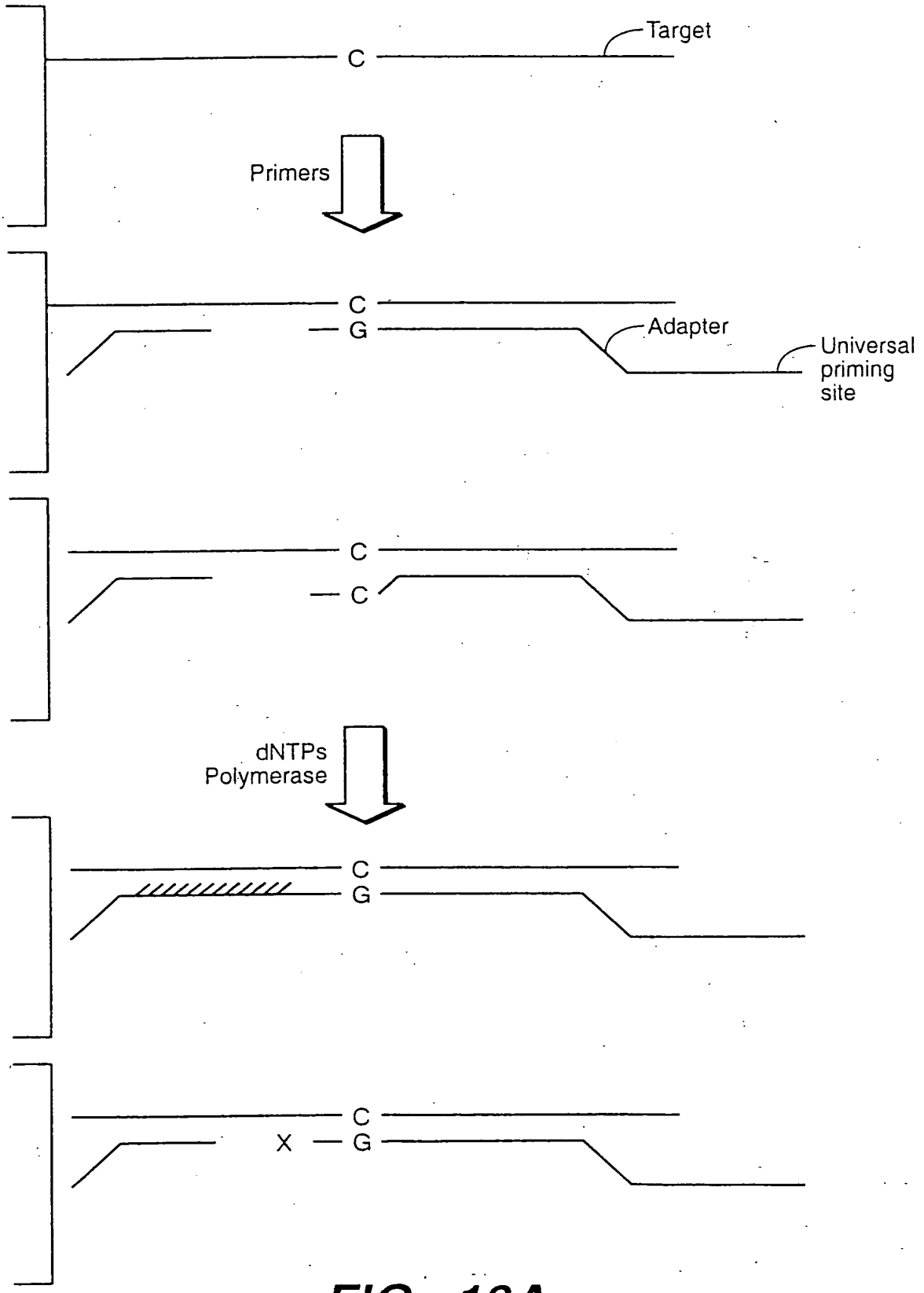

Target
C
Primers
C
G
Adapter
Universal priming site
C
C
dNTPs Polymerase
C
G
C
X
G

FIG._13A

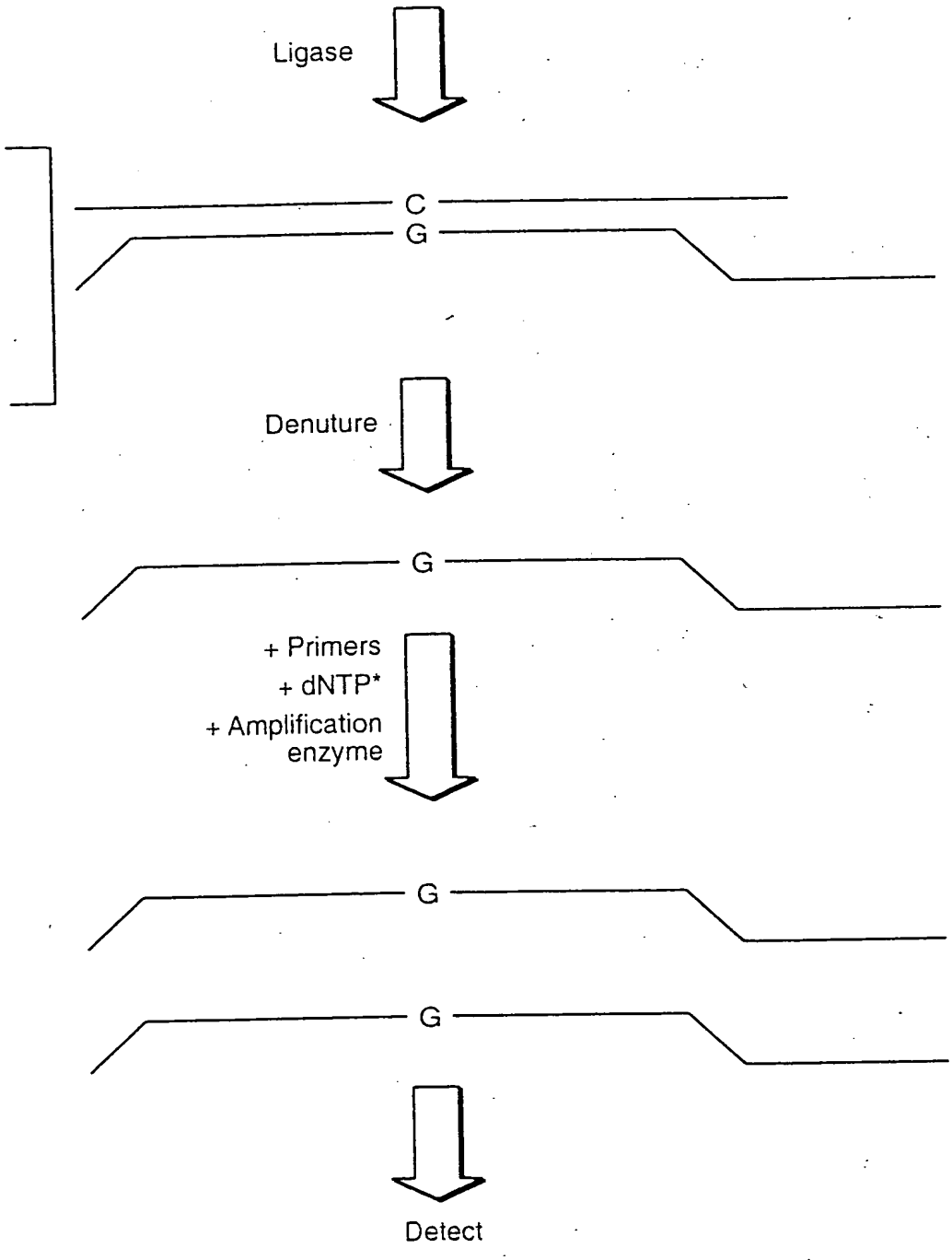

Ligase
C
G
Denuture
G
+ Primers
+ dNTP*
+ Amplification enzyme
G
G
Detect

FIG._13B

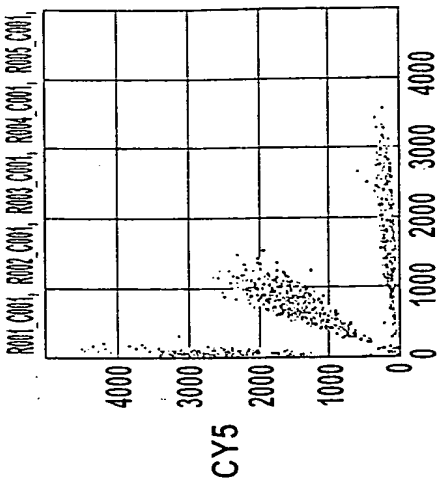


Fig. 14C

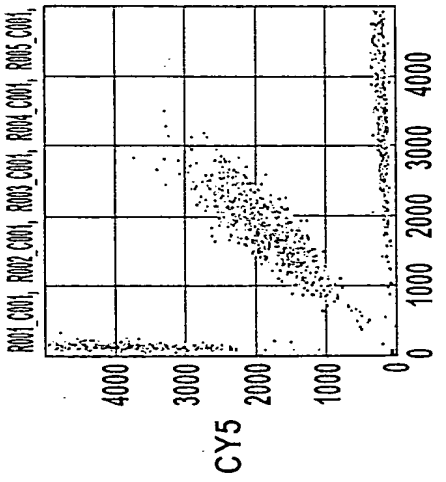


Fig. 14F

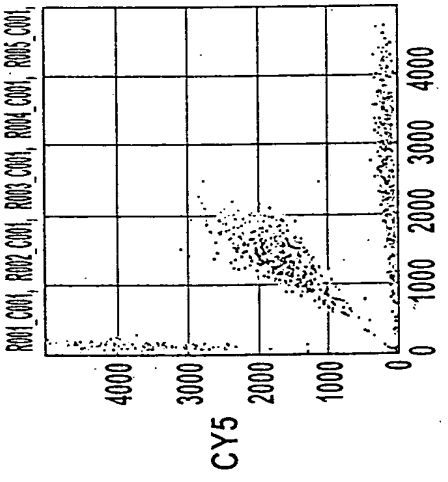


Fig. 14B

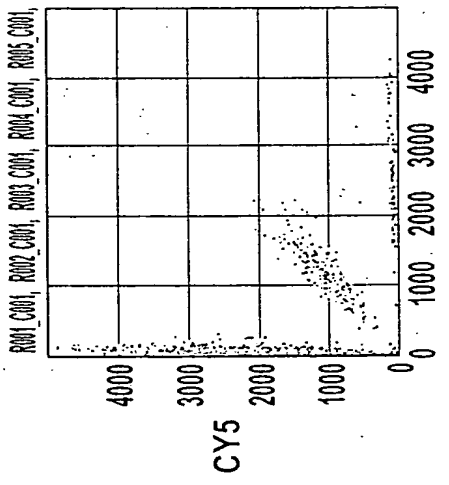


Fig. 14E

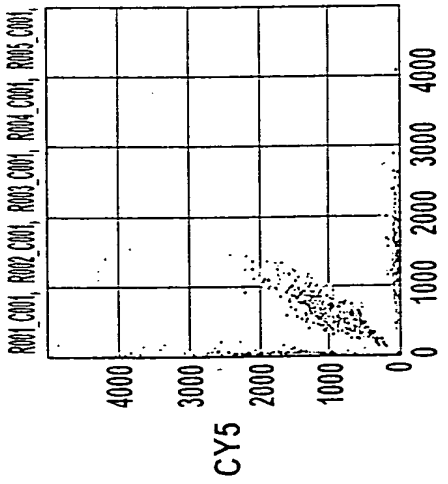


Fig. 14A

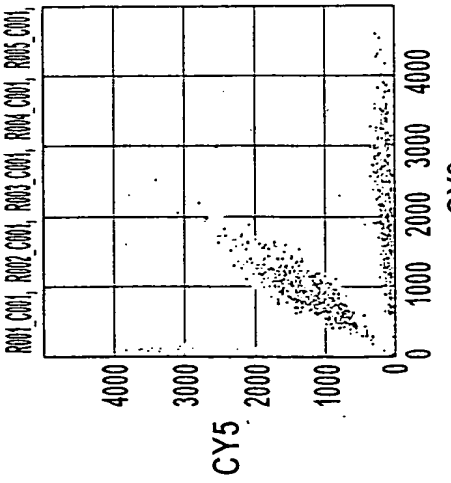


Fig. 14D

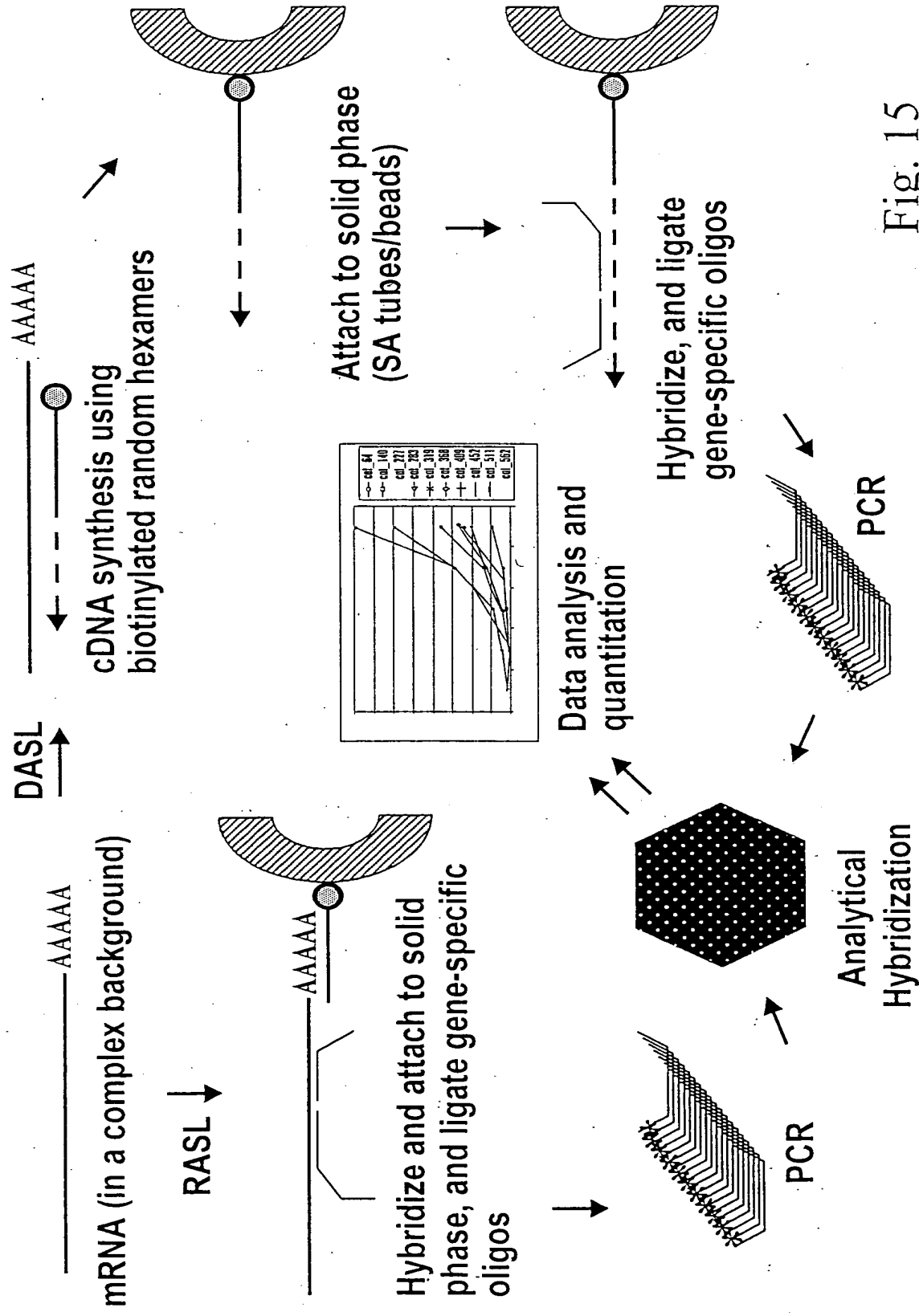

mRNA (in a complex background)
AAAAA
RASL
Hybridize and attach to solid phase, and ligate gene-specific oligos
AAAAA
PCR
Analytical Hybridization
Data analysis and quantitation
DASL
cDNA synthesis using biotinylated random hexamers
AAAAA
Attach to solid phase (SA tubes/beads)
Hybridize, and ligate gene-specific oligos
PCR

Fig. 15

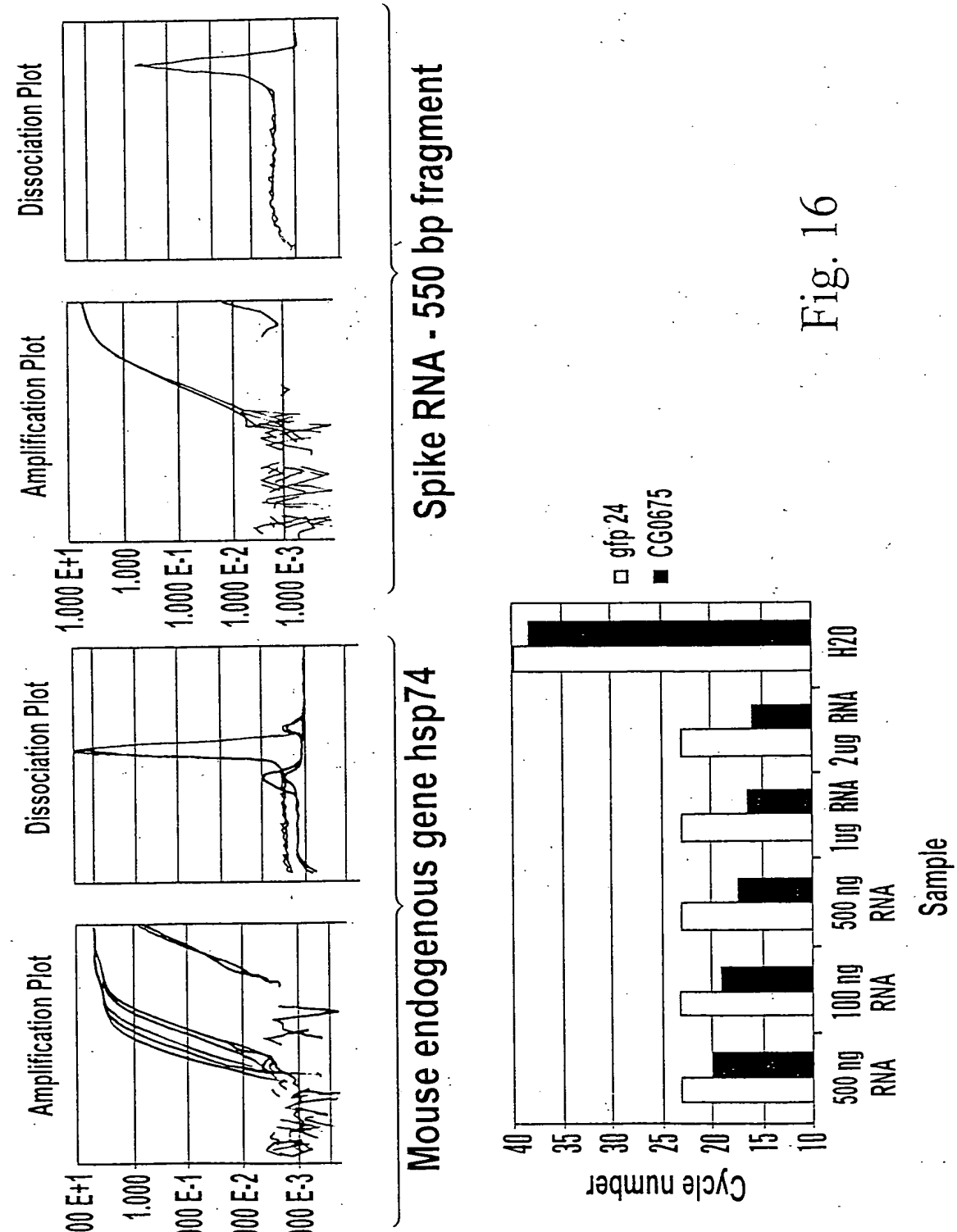

Amplification Plot
1.000 E+1
1.000
1.000 E-1
1.000 E-2
1.000 E-3
Dissociation Plot
Mouse endogenous gene hsp74
Amplification Plot
Dissociation Plot
Spike RNA - 550 bp fragment
gfp 24
CG0675
Cycle number
40
35
30
25
20
15
10
500 ng RNA
100 ng RNA
500 ng RNA
1ug RNA
2ug RNA
H20
Sample

Fig. 16

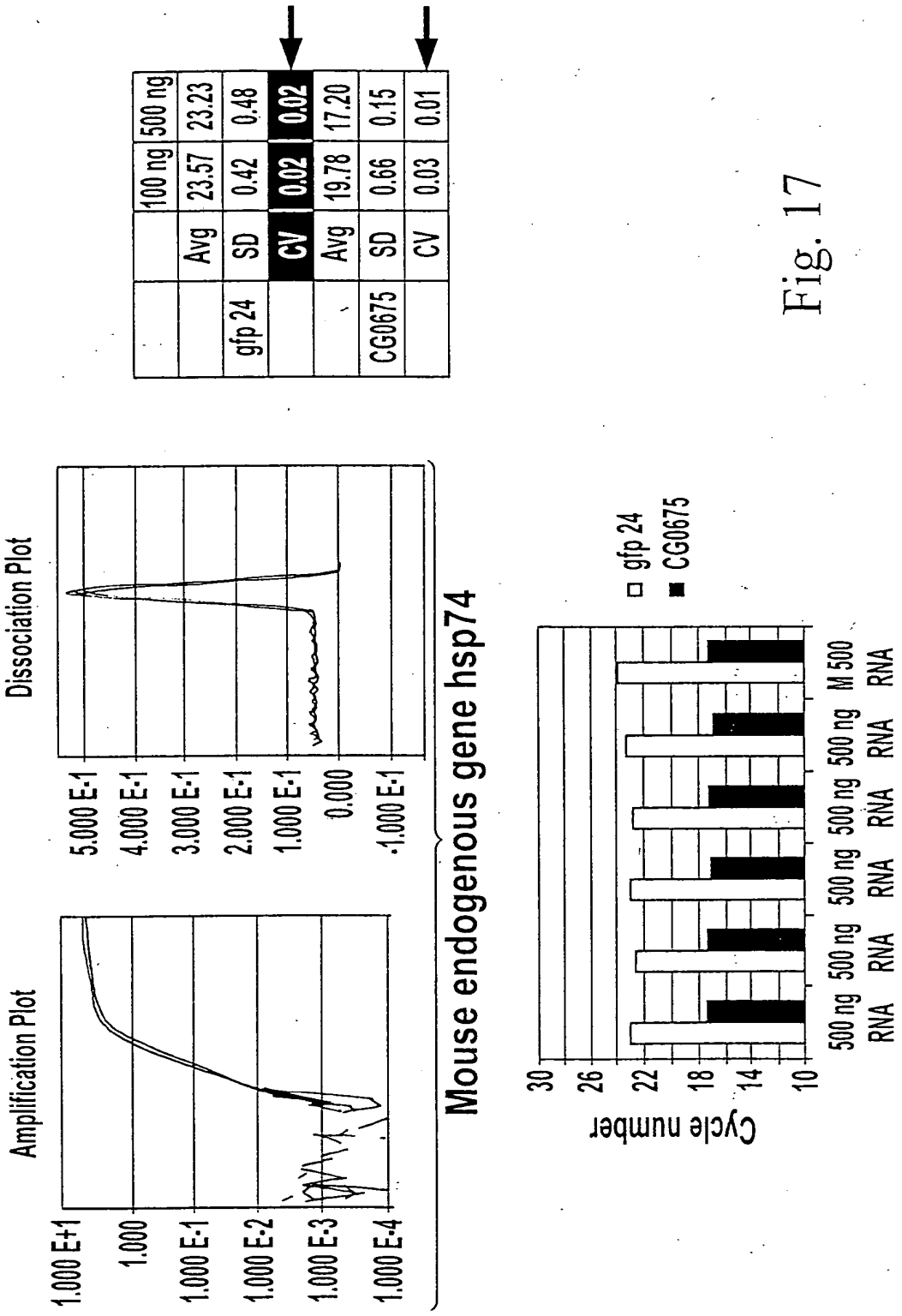

100 ng
500 ng
Avg
23.57
23.23
SD
0.42
0.48
CV
0.02
0.02
gfp 24
Avg
19.78
17.20
SD
0.66
0.15
CG0675
CV
0.03
0.01
Dissociation Plot
5.000 E-1
4.000 E-1
3.000 E-1
2.000 E-1
1.000 E-1
0.000
-1.000 E-1
Amplification Plot
1.000 E+1
1.000
1.000 E-1
1.000 E-2
1.000 E-3
1.000 E-4
Mouse endogenous gene hsp74
gfp 24
CG0675
30
26
22
18
14
10
Cycle number
500 ng RNA
500 ng RNA
500 ng RNA
500 ng RNA
500 ng RNA
M 500 RNA
Samples

Fig. 17

	pool 1	pool 2	pool 3	pool 4	pool 5	pool 6	pool 7	pool 8
cat	0.00E+00	1.00E+04	3.00E+04	1.00E+05	3.00E+05	1.00E+06	3.00E+06	1.00E+07
cre	1.00E+04	3.00E+04	1.00E+05	3.00E+05	1.00E+06	3.00E+06	1.00E+07	0.00E+00
E1A	3.00E+04	1.00E+05	3.00E+05	1.00E+06	3.00E+06	1.00E+07	0.00E+00	1.00E+04
GFP	1.00E+05	3.00E+05	1.00E+06	3.00E+06	1.00E+07	0.00E+00	1.00E+04	3.00E+04
gus	3.00E+05	1.00E+06	3.00E+06	1.00E+07	0.00E+00	1.00E+04	3.00E+04	1.00E+05
lacZ	1.00E+06	3.00E+06	1.00E+07	0.00E+00	1.00E+04	3.00E+04	1.00E+05	3.00E+05
luc	3.00E+06	1.00E+07	0.00E+00	1.00E+04	3.00E+04	1.00E+05	3.00E+05	1.00E+06
neo	1.00E+07	0.00E+00	1.00E+04	3.00E+04	1.00E+05	3.00E+05	1.00E+06	3.00E+06
bla	3.00E+05	3.00E+05	3.00E+05	3.00E+05	3.00E+05	3.00E+05	3.00E+05	3.00E+05
GST	3.00E+05	3.00E+05	3.00E+05	3.00E+05	3.00E+05	3.00E+05	3.00E+05	3.00E+05

Fig. 18

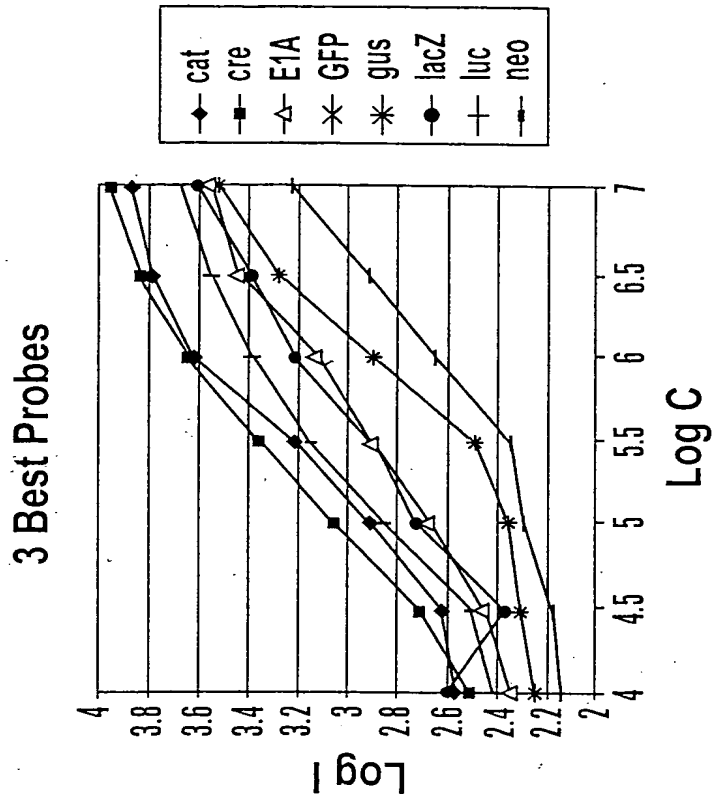


Fig. 19B

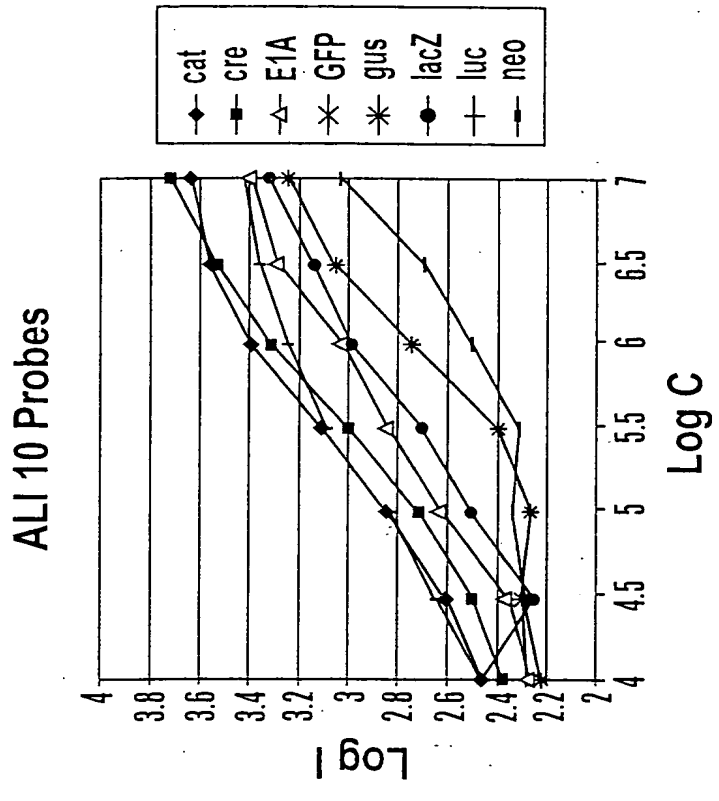


Fig. 19A

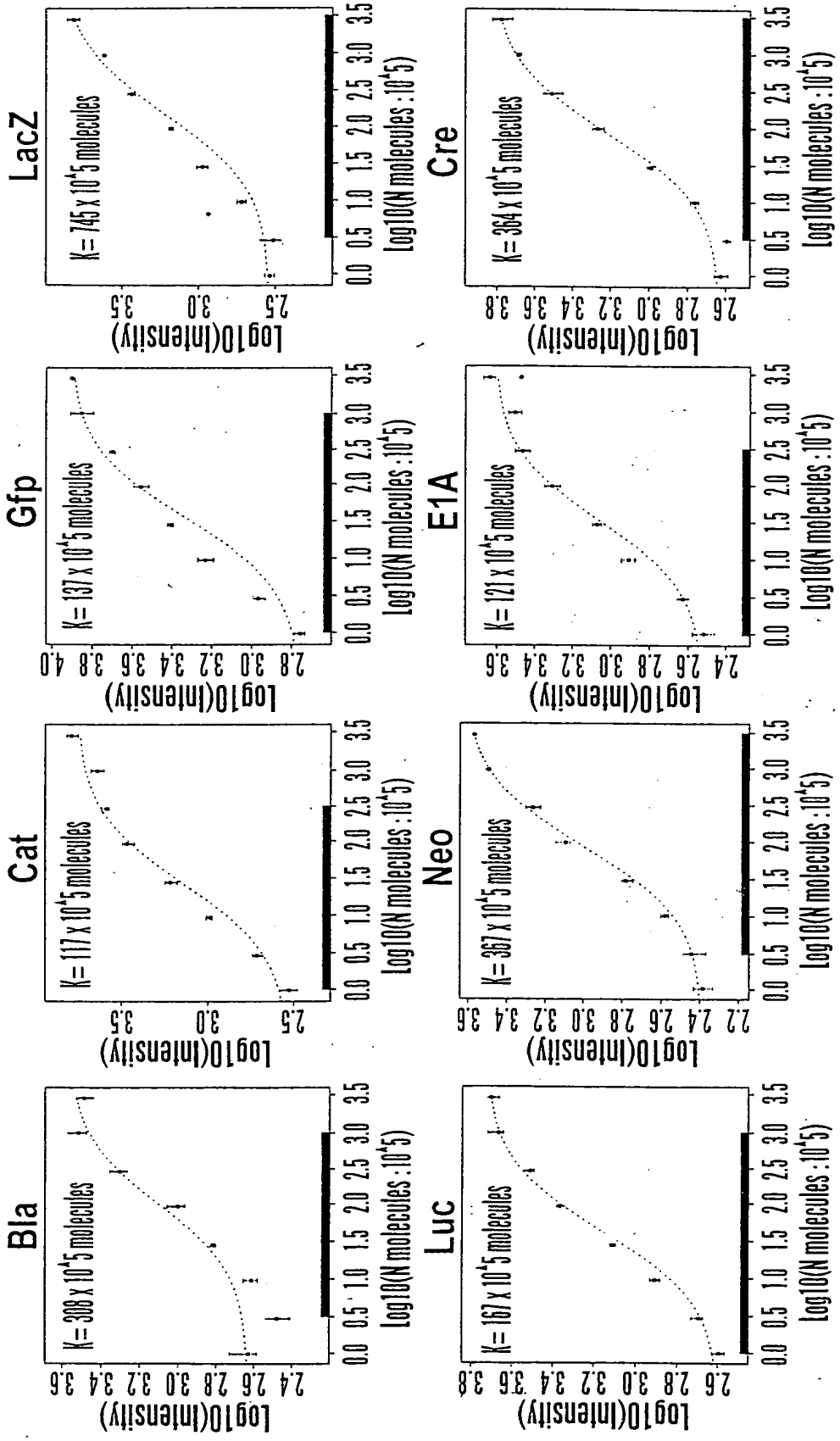
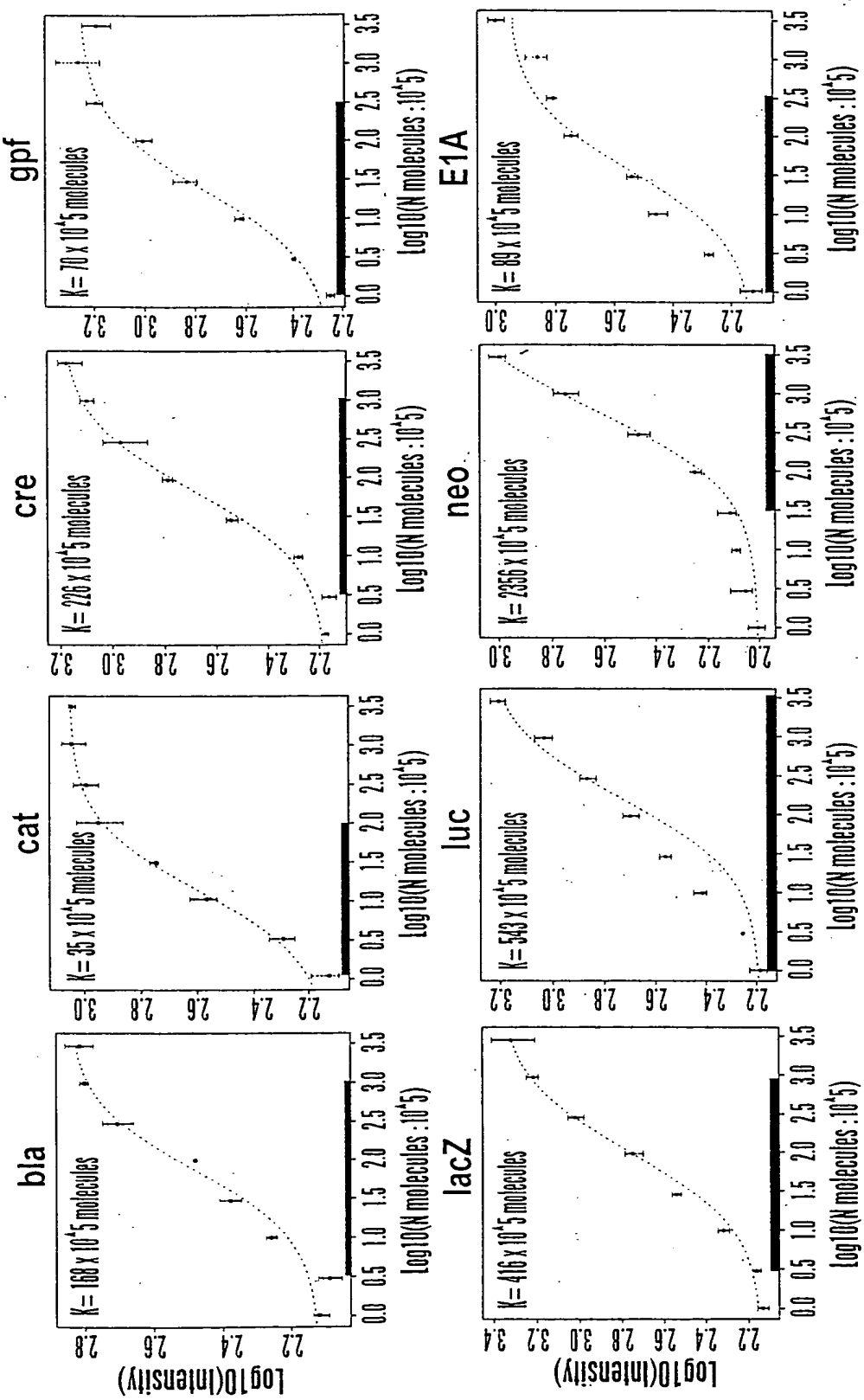
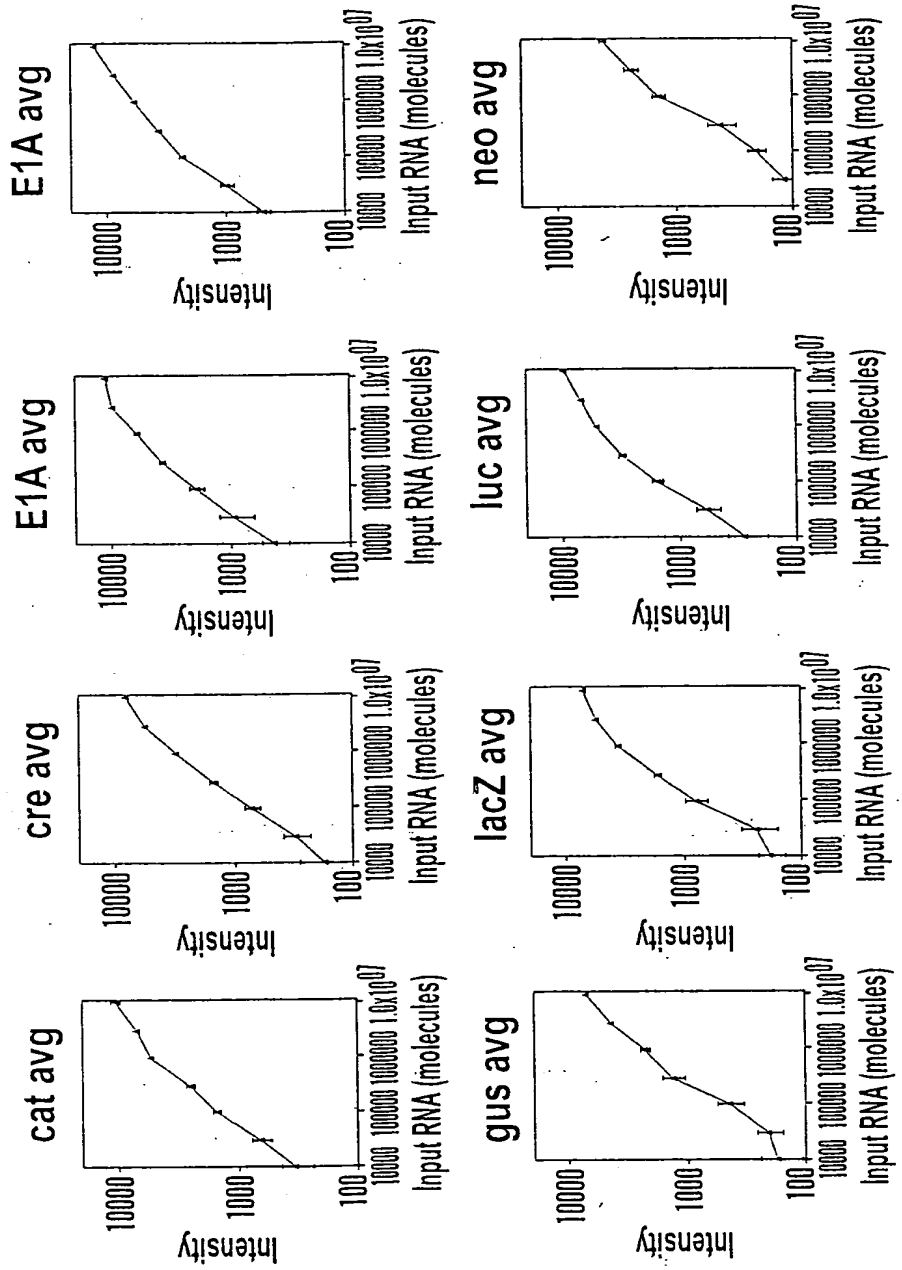

Bla
K= 308 x 10^-5 molecules
Cat
K= 117 x 10^-5 molecules
Gfp
K= 137 x 10^-5 molecules
LacZ
K= 745 x 10^-5 molecules
Luc
K= 167 x 10^-5 molecules
Neo
K= 367 x 10^-5 molecules
E1A
K= 121 x 10^-5 molecules
Cre
K= 364 x 10^-5 molecules
log10(Intensity)
log10(N molecules :10^-5)
3 fold detection range
• Error bars represent the range of intensities of 4 replicates.

Fig. 20



- 250 ng of total RNA/sample
- Ds DNA hybridization
- Error bars represent the range of intensities of 4 replicates.

Fig. 21



- 100 ng total RNA background, 12 replicates, 238-plex.
- All pre-PCR and post-PCR processes identical to SciOps including single stranded product hybridization to arrays.
- Dynamic range: 2.5 - 3 logs; Precision: better than 3 fold change.

Fig. 22

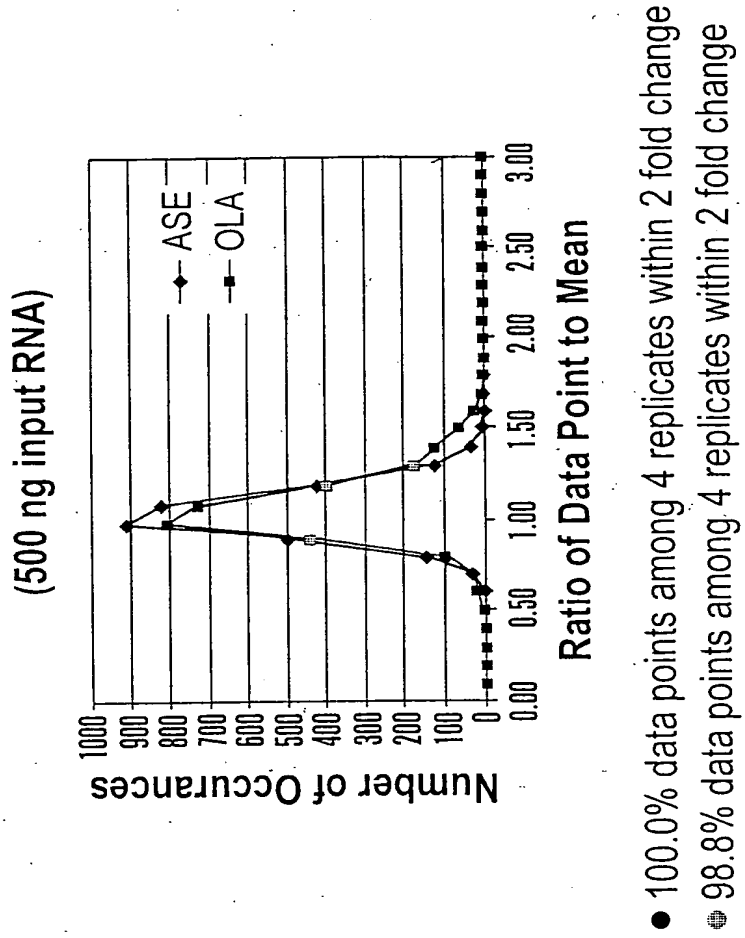

(500 ng input RNA)
Number of Occurances
1000
900
800
700
600
500
400
300
200
100
0
0.00
0.50
1.00
1.50
2.00
2.50
3.00
Ratio of Data Point to Mean
ASE
OLA
100.0% data points among 4 replicates within 2 fold change
98.8% data points among 4 replicates within 2 fold change

Fig. 23

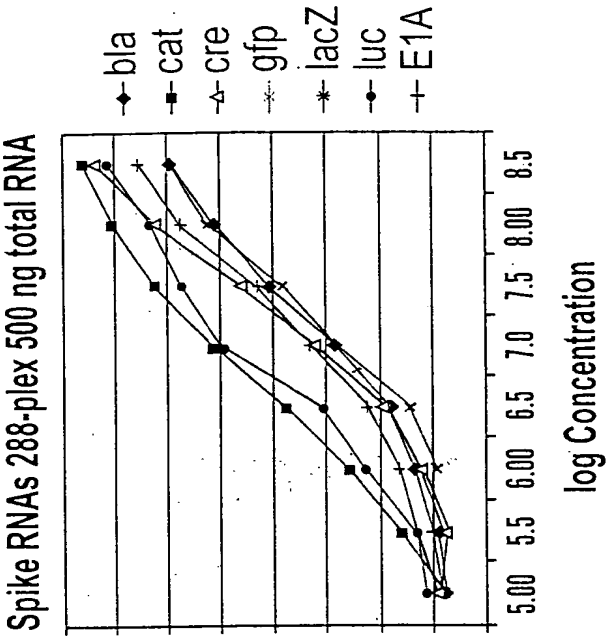


Fig. 24B

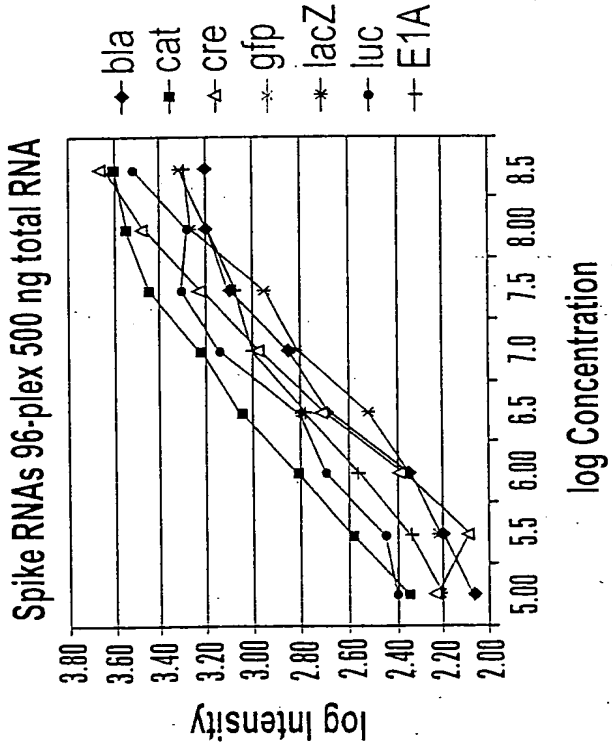


Fig. 24A

Fig. 25A

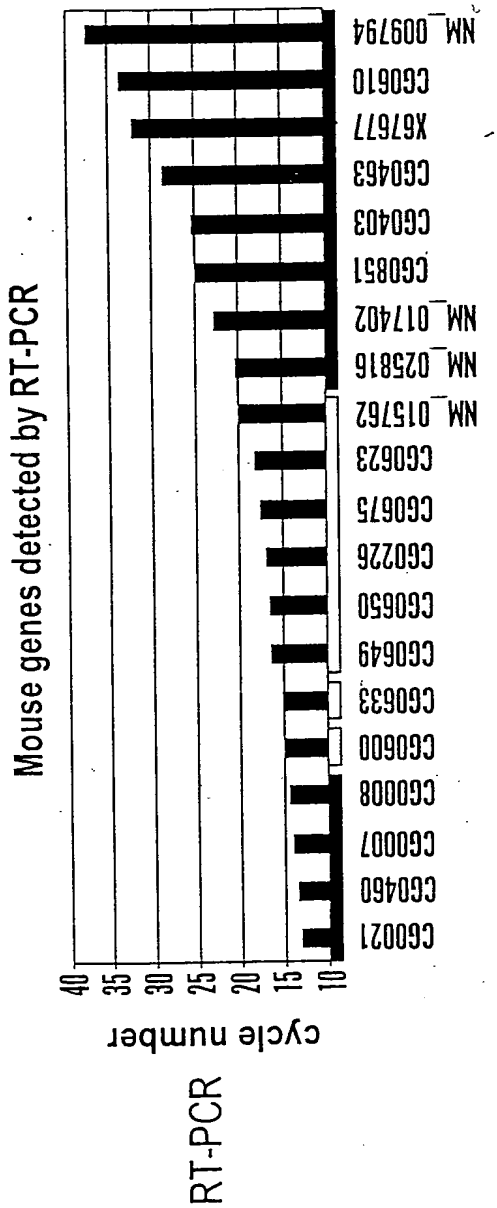
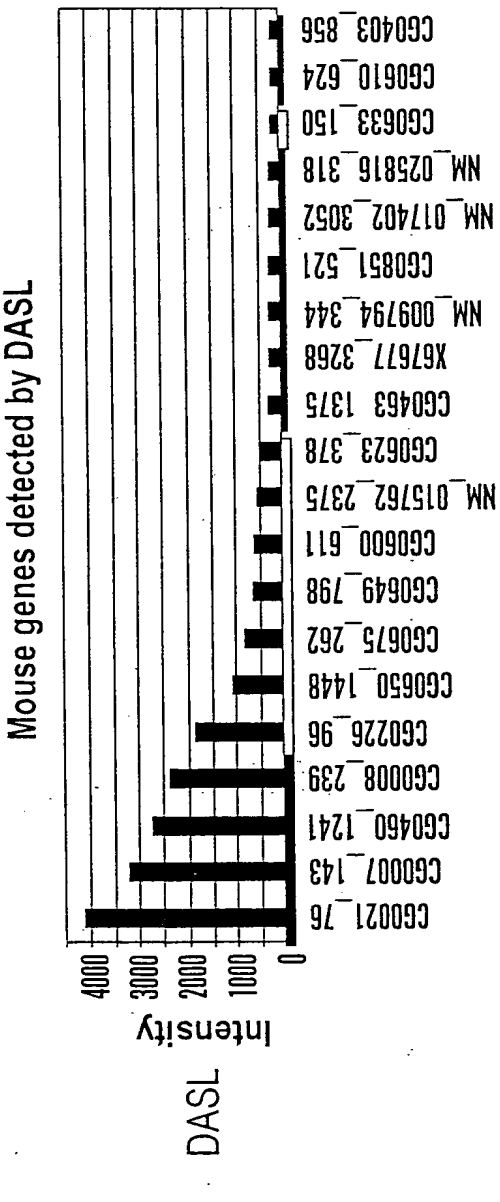


Fig. 25B



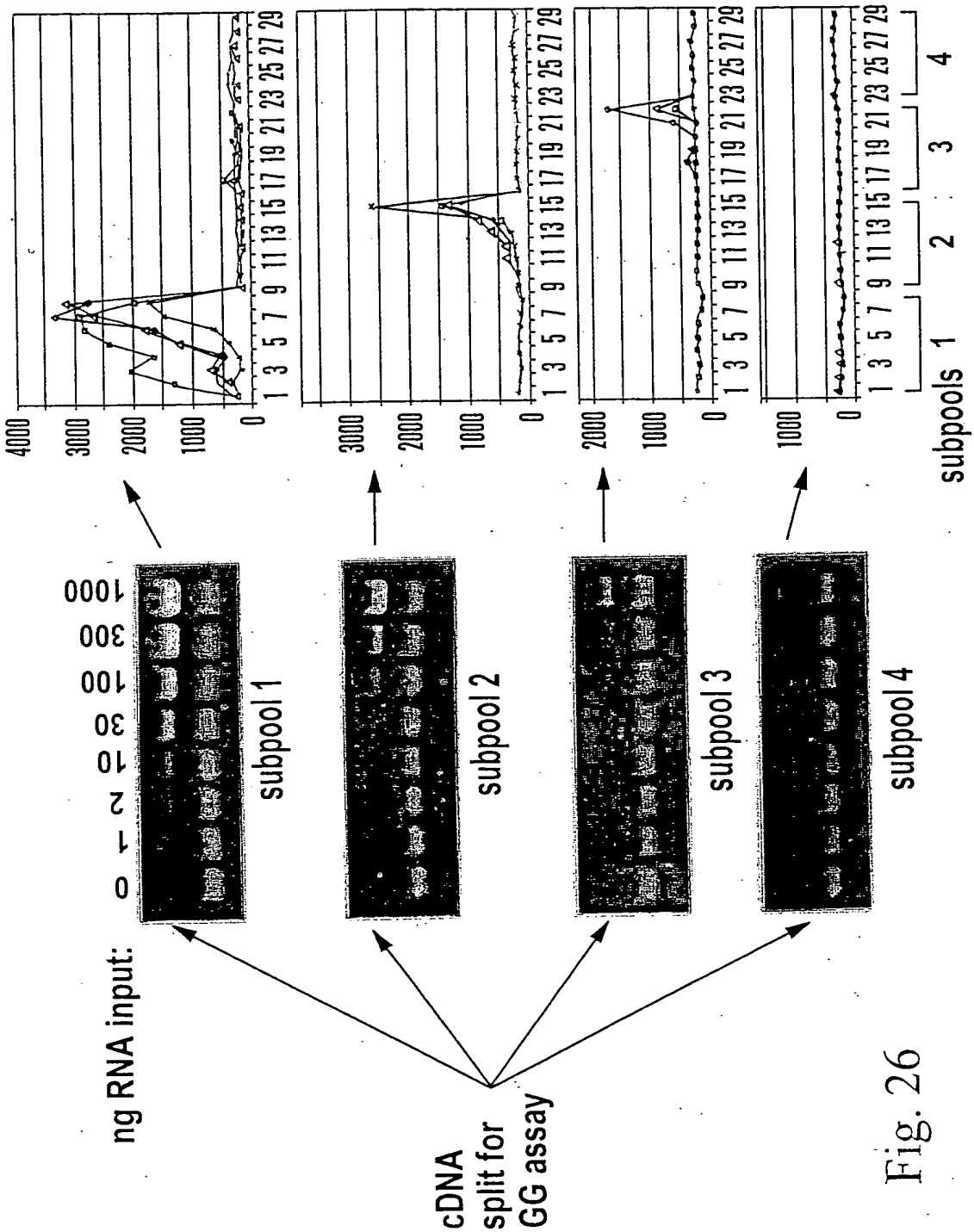

ng RNA input:
0
1
2
10
30
100
300
1000
cDNA split for GG assay
subpool 1
subpool 2
subpool 3
subpool 4
subpools 1 2 3 4
4000
3000
2000
1000
0
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29

Fig. 26

Fig. 27B

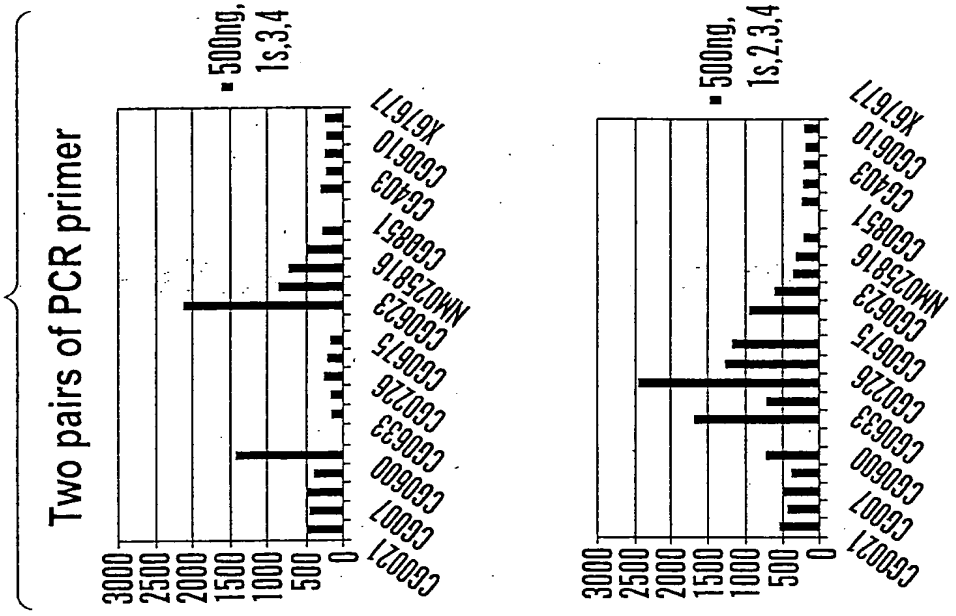


Fig. 27A